

What is claimed is:

1. A fluorescence observation apparatus comprising:

a light source;

an illumination optical system conducting irradiation light from the light source to a specimen;

an aperture member provided in the illumination optical system;

a first wavelength selective member;

a light splitter deflecting the irradiation light to conduct the light to the specimen;

an objective lens interposed between the light splitter and the specimen;

a second wavelength selective member transmitting fluorescent light emanating from the specimen; and

a detecting device receiving the fluorescent light,

wherein the aperture member has a partial aperture through which part of the irradiation light passes, and the fluorescence observation apparatus is provided with a projection optical system projecting the aperture member at a pupil position of the objective lens so that a size of the partial aperture and a magnification of the projection optical system are set to satisfy the following Conditions:

$$0.5NA \leq NA_1 < NA$$

$$NA_1 < n$$

where NA_1 is a numerical aperture derived from an angle made by a ray closest to an optical axis, of rays of light passing through the partial aperture, with the optical axis on the specimen, NA is a maximum numerical aperture of the objective lens, and n is a refractive index of a medium holding the specimen.

2. A fluorescence observation apparatus comprising:

a light source;

an illumination optical system conducting irradiation light from the light source to a

specimen;

- 5 an aperture member provided in the illumination optical system;
- a first wavelength selective member;
- a light splitter deflecting the irradiation light to conduct the light to the specimen;
- an objective lens interposed between the light splitter and the specimen;
- a second wavelength selective member transmitting fluorescent light emanating from
- 10 the specimen; and
- a detecting device receiving the fluorescent light,

wherein the aperture member has a partial aperture through which part of the irradiation light passes, and the fluorescence observation apparatus is provided with a projection optical system projecting the aperture member at a pupil position of the objective

15 lens so that a size of the partial aperture and a magnification of the projection optical system are set to satisfy the following Conditions:

$$0.5NA \leq NA_1 < 0.95NA$$

$$NA_1 < NA_2 \leq NA$$

where NA_1 is a numerical aperture derived from an angle made by a ray closest to an

20 optical axis, of rays of light passing through the partial aperture, with the optical axis on the specimen, NA is a maximum numerical aperture of the objective lens, and NA_2 is a numerical aperture derived from an angle made by a ray farthest from the optical axis, of rays of light passing through the partial aperture, with the optical axis on the specimen.

3. A fluorescence observation apparatus comprising:

- a light source;
- an illumination optical system conducting irradiation light from the light source to a
- specimen;
- 5 an aperture member provided in the illumination optical system;
- a first wavelength selective member;
- a light splitter deflecting the irradiation light to conduct the light to the specimen;

an objective lens interposed between the light splitter and the specimen;

a second wavelength selective member transmitting fluorescent light emanating from the specimen; and

a detecting device receiving the fluorescent light,

wherein the aperture member has a partial aperture through which part of the irradiation light passes, and the fluorescence observation apparatus is provided with a projection optical system projecting the aperture member at a pupil position of the objective lens so that a size of the partial aperture and a magnification of the projection optical system are set to satisfy the following Conditions:

$$0.75NA \leq NA_1 < NA$$

$$NA_1 < n$$

where NA_1 is a numerical aperture derived from an angle made by a ray closest to an optical axis, of rays of light passing through the partial aperture, with the optical axis on the specimen, NA is a maximum numerical aperture of the objective lens, and n is a refractive index of a medium holding the specimen.

4. A fluorescence observation apparatus according to any one of claims 1-3, wherein a shape of the partial aperture is annular.

5. A fluorescence observation apparatus according to claim 2, further satisfying the following condition:

$$NA_2 < n$$

where n is a refractive index of a medium holding the specimen.

6. A fluorescence observation apparatus according to claim 1, further satisfying the following condition:

$$0.1 \leq NA_2 - NA_1$$

where NA_2 is a numerical aperture derived from an angle made by a ray farthest from

5 the optical axis, of rays of light passing through the partial aperture, with the optical axis on the specimen.

7. A fluorescence observation apparatus according to claim 3 or 5, wherein the objective lens has an numerical aperture above 1.0.

8. A fluorescence observation apparatus according to claim 3 or 5, wherein the objective lens has an numerical aperture above 1.35.

9. A fluorescence observation apparatus according to any one of claims 1-3, wherein the aperture member is constructed to be movable in and out of the illumination optical system.

10. A fluorescence illumination apparatus comprising:

a light source;

an illumination optical system conducting irradiation light from the light source to a specimen;

5 an aperture member provided in the illumination optical system;

a first wavelength selective member; and

a light splitter deflecting the irradiation light to conduct the light to the specimen,

wherein the aperture member has a partial aperture through which part of the irradiation light passes, and the fluorescence illumination apparatus is provided with a projection optical system projecting the aperture member at a pupil position of the objective lens so that a size of the partial aperture and a magnification of the projection optical system are set to satisfy the following Conditions:

$$0.5NA \leq NA_1 < NA$$

$$NA_1 < n$$

15 where NA_1 is a numerical aperture derived from an angle made by a ray closest to an

optical axis, of rays of light passing through the partial aperture, with the optical axis on the specimen, NA is a maximum numerical aperture of the objective lens, and n is a refractive index of a medium holding the specimen.